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UNCLASSIFIED

TECHNICAL MANUSCRIPT 79

ENHANCEMENT OF STAINING INTENSITY IN THE FLUORESCENT ANTIBODY REACTION

AUGUST 1963

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

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ENCHANCEMENT OF STAINING INTENSITY IN THE FLUORESCENT ANTIBODY REACTION

The work reported here was performed under Project 4B11-05-014, "Research on BW Rapid Warning Systems," Task -02, Biological Principles of BW Detection. Expenditure order was 2018. This material was originally submitted as manuscript 5171.

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Project 1C022301A07102 July 1963

DDC AVAILABILITY NOTICE

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Charles E. Hudak.

ABSTRACT

A method has been developed for enhancing the staining intensity of bacterial smears treated with fluorescent antibody. The procedure involved the substitution of carbonate-bicarbonate buffer pH 9.0 for phosphate-buffered saline pH 7.2 in the washing of slide preparations and in buffering the glycerol mounting medium. A marked increase in fluorescent brightness was obtained with all dilutions of homologous conjugate employed with three test strains (*Bacillus anthracis*, *Brucella abortus*, and *Serratia marcescens*).

ENHANCEMENT OF STAINING INTENSITY IN THE FLUORESCENT ANTIBODY REACTION

It is known that fluorescein produces strong fluorescence in alkaline solutions[1] and that a marked increase to maximal intensity occurs at approximately pH 9.0 to 12.0.[2,3] Solutions prepared in carbonate-bicarbonate buffer pH 9.6 and in 0.1N sodium hydroxide possess the same fluorescence efficiency, but a more stable solution is obtained with carbonate buffer.[3] This study is concerned with evaluating the effect of alkalinity (pH 9.0) on staining brightness in the fluorescent antibody reaction. The procedure most frequently employed consists of washing stained smears in phosphate-buffered saline pH 7.2 and mounting in buffered glycerol of the same or slightly higher pH. Initial studies with bacterial smears show that stained preparations washed and mounted in buffered solutions pH 9.0 exhibit a marked increase in fluorescent brilliance. Staining intensity at pH 9.6 is not significantly different from that observed at pH 9.0.

The following strains were tested for staining reactions: Bacillus anthracis CD-3S, Brucella abortus CD-476, and Serratia marcescens 8UK. Immune sera were obtained from white New Zealand rabbits immunized over a three-week period. Immune and normal sera were fractionated by the methanol procedure of Dubert et al.[4] The method employed for conjugating globulin was essentially that of Marshall et al[5] with the following exceptions: (a) 0.04 milligram of crystalline fluorescein isothiocyanate (BBL) per milligram of protein was used, and (b) untagged dye was removed with a Sephadex column (G-25, medium grade) equilibrated and eluted with physiological saline according to the method of Gordon et al.[6] Air-dried and gently heat-fixed smears were prepared from live suspensions of the test strains grown on appropriate media. Preparations were stained with homologous conjugate for 20 minutes in a moist chamber at room temperature. After staining, the slides were rinsed and washed in carbonate-bicarbonate buffer (0.5M, pH 9.0) for 10 minutes in a Coplin jar and then wiped free of excess buffer. The smears were mounted in glycerol adjusted to pH 9.0 with carbonate-bicarbonate buffer. Stock preparations of buffered glycerol and carbonate buffer were stored at 4°C and the pH again tested prior to use in experimental studies. Slides were examined with a Zeiss Fluorescence microscope equipped with dark field condenser and OSRAM HBO 200 mercury vapor lamp. Filters consisted of Schott BG-12 exciter filter in combination with barrier filters BG-23 and GG-4.

The effect of alkalinity on the fluorescent antibody reaction is presented in Table I. High-intensity fluorescence was obtained with lower dilutions of each conjugate. In all dilutions producing a staining reaction, the fluorescence was distinctly brighter at pH 9.0 than at pH 7.2. Moreover, endpoint dilutions exhibiting detectable staining were higher at pH 9.0.

The experimental findings presented in this report may have practical application in several important aspects: (a) upgrading reactions of low fluorescence, (b) preparing higher working dilutions of conjugate with possible reduction of low-titer cross-reacting antibodies, and (c) economy of working materials.

TABLE I. FLUORESCENT INTENSITY OF STAINED SMEARS WASHED IN CARBONATE-BICARBONATE BUFFER pH 9.0 AND IN BUFFERED SALINE pH 7.2 (MOUNTED IN BUFFERED GLYCEROL OF CORRESPONDING pH)

Test Strain	pH	Undiluted b/	Fluorescent Intensity of Homologous Conjugate a/ 1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Normal Globulin Conjugate (1:4)	Unstained Smears
B. anthracis	9.0	H4+	H4+	H4+	H4+	H4+	4+	3+	3+	3+	1+	-	-	-
	7.2	4+	4+	4+	4+	3+	2+	1+	1+	-	-	-	-	-
B. abortus	9.0	H4+	H4+	H4+	H4+	H4+	H4+	H4+	3+	2+	-	-	-	-
	7.2	4+	4+	4+	4+	4+	4+	4+	2+	-	-	-	-	-
S. marcescens	9.0	H4+	H4+	H4+	H4+	H4+	H4+	4+	4+	4+	2+	-	-	-
	7.2	4+	4+	4+	3+	3+	3+	2+	2+	1+	-	-	-	-

a. Microscopic reactions graded by the conventional method for estimating fluorescence: 1+ to H4+, minimal to maximal intensity (H4+, High intensity fluorescence, more brilliant than the usual 4+); - , no fluorescence.

b. Dilutions of conjugate prepared in phosphate-buffered saline pH 7.2; standard macroscopic tube agglutination tests showed a titer of 1:1280 for both B. abortus and S. marcescens conjugates.

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